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New publications in the PARATUBERCULOSIS database (1512-1533)

1512 Ignatov, D. , Malakho, S., Majorov, K., Skvortsov, T., Apt, A., Azhikina, T.

RNA-Seq Analysis of Mycobacterium avium Non-Coding Transcriptome
Plos One, (2013) 8, Article Number: e74209 DOI: 10.1371/journal.pone.0074209
Published: SEP 16 2013-Deep sequencing was implemented to study the transcriptional landscape of Mycobacterium avium. High-resolution transcriptome analysis identified the transcription start points for 652 genes. One third of these genes represented leaderless transcripts, whereas the rest of the transcripts had 5' UTRs with the mean length of 83 nt. In addition, the 5' UTRs of 6 genes contained SAM-IV and Ykok types of riboswitches. 87 antisense RNAs and 10 intergenic small RNAs were mapped. 6 intergenic small RNAs, including 4.5S RNA and rnpB, were transcribed at extremely high levels. Although several intergenic sRNAs are conserved in M. avium and M. tuberculosis, both of these species have unique intergenic sRNAs. Moreover, we demonstrated that even conserved small RNAs are regulated differently in these species. Different sets of intergenic sRNAs may underlie differences in physiology between conditionally pathogenic M. avium and highly specialized pathogen M. tuberculosis.

1513 Swift, B.M.C., Denton, E.J., Mahendran, S.A., Huxley, J.N., Rees, C.E.D.

Development of a rapid phage-based method for the detection of viable Mycobacterium avium subsp paratuberculosis in blood within 48 h
Journal of Microbiological Methods, (2013) 94, 175- 179
The aim of this study was to develop a methodology to rapidly detect viable Mycobacterium avium subsp. paratuberculosis (MAP) in clinical blood samples. MAP cells spiked into commercially available blood were recovered using optimised peptide-mediated magnetic separation (PMMS) and detected using a phage-based method, and the identity of the cells detected confirmed using nested-PCR amplification of MAP signature sequences (IS900). The limit of detection was determined to be 10 MAP cells per ml of blood and was used to detect MAP present in clinical bovine blood samples. Using the PMMS-phage method there was no difference when detecting MAP from whole blood or from isolated buffy coat. MAP was detected in animals that were milk-ELISA positive (15 animals) by PMMS-phage and no MAP was detected in blood samples from an accredited Johne's disease free herd (5 animals). In a set of samples from one herd (10 animals) that came from animals with variable milk ELISA status, the PMMS-phage results agreed with the positive milk-ELISA results in all but one case. These results show that the PMMS-phage method can detect MAP present in naturally infected blood. Total assay time is 48 h and, unlike PCR-based detection tests, only viable cells are detected. A rapid method for detecting MAP in blood could further the understanding of disseminated infection in animals with Johne's disease.

1514 Sarno, E., Keller, S., Wittenbrink, M.M., Stephan, R.

Occurrence of Mycobacterium avium subsp paratuberculosis in fecal samples of hunted deer, chamois and ibex in Switzerland
Schweizer Archiv fur Tierheilkunde, (2013) 155, 523-525
Abstract not available. Full paper in PDF will not be available. Use your library, please.

1515 Goodridge, A., Correa, R., Castro, P., Escobar, C., de Waard, J.H.

Serum samples can be substituted by plasma samples for the diagnosis of paratuberculosis
Preventive Veterinary Medicine, (2013) 112, 147-149
Employing plasma samples rather than serum samples for serological paratuberculosis diagnosis is practical, especially when bovine TB is assessed in the same cattle herd with the gamma interferon bovine avian (IFN-gamma BA) test. We demonstrate that antibody titers in serum and plasma samples, utilizing the PARACHECK (R) ELISA kit, are highly comparable (Cohen's kappa test, k=0.955). We conclude that serum can be replaced with plasma in this commercially available antibody detection assay resulting in working hour savings for sampling and blood sample work-up and cost reductions for materials and sample storage.

1516 Moron-Cedillo, F.D., Cortez-Romero, C., Gallegos-Sanchez, J., Figueroa-Sandoval, B., quino-Perez, G., mante-Orozco, A.

Prevalence of Infection by Mycobacterium avium Subspecie paratuberculosis in Flocks of Sheep of Two Regions of San Luis Potosi, Mexico
Revista Cientifica-Facultad de Ciencias Veterinarias, (2013) 23, 293-299
Paratuberculosis (Ptb), also known as Johne's disease, is caused by Mycobacterium avium subspecie paratuberculosis (Map). The disease is contagious in cattle, sheep, goats and other ruminants; in humans it is associated with Crohn's disease. Progressive weight loss is observed in sheep, and in advanced stages, clumped stools and diarrhea. To diagnose this disease there are different tests: one is using the feces, from which an insertion sequence 900 (IS900) Map genome is obtained, making possible the organism identification through a Polymerase Chain Reaction (PCR), and a second one in blood serum through the Agar Gel Immunodiffusion test (AGID). These two techniques were selected to estimate the prevalence of Map. Fecal and blood samples of 211 asymptomatic sheep were analyzed in 32 flocks from 10 communities in Salinas and Villa of Ramos, San Luis Potosi. The Map prevalence per flock between communities with the AGID test ranged from 4.35 to 33.33%, with a mean of 9.48%. The prevalence with the nested PCR test came out with a value varying from 4.26 to 33.33% and a mean of 7.58% between communities. The AGID test detected 20 positive sheep to Map antibodies. The nested PCR detected 16 sheep excreting Map. By using the PCR test as the gold standard, the AGID test had a sensitivity of 81% and a specificity of 96%, with a kappa agreement value of 0.96. Being Map present in the Municipalities of Salinas and Villa de Ramos, San Luis Potosi, it is necessary to take the relevant measures to control and eradicate it, preventing its spread and economic losses in all livestock farms.

1517 Wu, Y.L., Ding, Y.P., Gao, J., Tanaka, Y., Zhang, W.

Risk Factors and Primary Prevention Trials for Type 1 Diabetes
International Journal of Biological Sciences , (2013) 9, 666-679
Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease resulting in the designated immune destruction of insulin producing beta-cells, usually diagnosed in youth, and associated with important psychological, familial, and social disorders. Once diagnosed,

patients need lifelong insulin treatment and will experience multiple disease-associated complications. There is no cure for T1DM currently. The last decade has witnessed great progress in elucidating the causes and treatment of the disease based on numerous researches both in rodent models of spontaneous diabetes and in humans. This article summarises our current understanding of the pathogenesis of T1DM, the roles of the immune system, genes, environment and other factors in the continuing and rapid increase in T1DM incidence at younger ages in humans. In addition, we discuss the strategies for primary and secondary prevention trials of T1DM. The purpose of this review is to provide an overview of this disorder's pathogenesis, risk factors that cause the disease, as well as to bring forward an ideal approach to prevent and cure the disorder.

1518 Hughes, V., Denham, S., Bannantine, J.P., Chianini, F., Kerr, K., May, L., McLuckie, J., Nath, M., Stevenson, K.

Interferon gamma responses to proteome-determined specific recombinant proteins: Potential as diagnostic markers for ovine Johne's disease
Veterinary Immunology and Immunopathology, (2013) 155, 197-204
Johne's disease (JD), or paratuberculosis is a fatal enteritis of animals caused by infection with Mycobacterium avium subspecies paratuberculosis (Map). There may be a long subclinical phase with no signs of clinical disease. Diagnosis of JD is problematic and no test can reliably detect sub-clinical disease. Th1 responses to Map are believed to be activated first with a later switch to Th2 responses and progression to clinical disease. Detection of a cell-mediated response, indicated by interferon gamma (IFN-gamma) produced in response to mycobacterial antigens, may give an early indication of infection. Crude extracts of Map (PPDj) have been used to detect the cell-mediated response, but more specific, quantifiable antigens would improve the test. Thirty Map-specific proteins were screened for their ability to raise a cell-mediated response in subclinically infected sheep. Four proteins were selected and tested using blood from subclinical animals and controls from a JD-free flock. Three proteins elicited IFN-gamma levels which were higher in the subclinical group than in the control group, two were statistically significant. Thus these proteins have the ability to discriminate groups of infected and uninfected animals and may have use in diagnosis of JD.

1519 Khalifeh, M.S., Stabel, J.R.

Clinical Disease Upregulates Expression of CD40 and CD40 Ligand on Peripheral Blood Mononuclear Cells from Cattle Naturally Infected with Mycobacterium avium subsp paratuberculosis
Clinical and Vaccine Immunology, (2013) 20, 1274-1282
CD40 and CD40 ligand (CD40L) have costimulatory effects as part of a complex series of events in host immunity. In this study, the expression of CD40 and CD40L on peripheral blood mononuclear cells (PBMCs) isolated from cattle with Johne's disease were measured on freshly isolated PBMCs and on cells cultured for 8, 24, and 72 h in the presence or absence of live Mycobacterium avium subsp. paratuberculosis and exogenous gamma interferon, interleukin 10, and transforming growth factor beta. Results demonstrated greater CD40 and CD40L expression on fresh PBMCs obtained from animals in the clinical stage of disease (symptomatic) than those from healthy control animals or cows in the subclinical stage of disease (asymptomatic). A similar expression profile with greater magnitude was noted for cultured PBMCs, with increased CD40 expression after 8 and 24 h of culture and increased CD40L expression between 24 and 72 h on PBMCs obtained from clinically infected animals. The addition of live M. avium subsp. paratuberculosis to cell cultures resulted in downregulation of CD40L expression in naturally infected cows, regardless of the disease stage. In contrast, the addition of live M. avium subsp. paratuberculosis to cultures resulted in upregulation of CD40 expression on cells obtained from clinically infected animals, while a

decrease in expression was noted for healthy and subclinically infected cows. No effects of exogenous cytokines on CD40 or CD40L expression were observed. These results clearly point for the first time to a disparity in the expression of these costimulatory molecules on immune cells from cattle in different stages of Johne's disease and suggest further investigation into their roles in paratuberculosis pathogenesis.

1520 Klanicova, B., Seda, J., Slana, I., Slany, M., Pavlik, I.

The Tracing of Mycobacteria in Drinking Water Supply Systems by Culture, Conventional, and Real Time PCRs
Current Microbiology, (2013) 67, 725-731
Mycobacteria are widely present in diverse aquatic habitats, where they can survive for months or years while some species can even proliferate. The resistance of different mycobacterial species to disinfection methods like chlorination or ozonation could result in their presence in the final tap water of consumers. In this study, the culture method, Mycobacterium tuberculosis complex conventional duplex PCR for detection of non-tuberculous mycobacteria (NTM) and quantitative real-time PCR (qPCR) to detect three subspecies of M. avium species (M. a. avium, M. a. hominissuis, and M. a. paratuberculosis) were used to trace their possible path of transmission from the watershed through the reservoir and drinking water plant to raw drinking water and finally to households. A total of 124 samples from four drinking water supply systems in the Czech Republic, 52 dam sediments, 34 water treatment plant sludge samples, and 38 tap water household sediments, were analyzed. NTM of 11 different species were isolated by culture from 42 (33.9 %) samples; the most prevalent were M. gordonae (16.7 %), M. triplex (14.3 %), M. lentiflavum (9.5 %), M. a. avium (7.1 %), M. montefiorenase (7.1 %), and M. nonchromogenicum (7.1 %). NTM DNA was detected in 92 (76.7 %) samples. By qPCR analysis a statistically significant decrease ($P < 0.01$) was observed along the route from the reservoir (dam sediments), through water treatment sludge and finally to household sediments. The concentrations ranged from 10(0) to 10(4) DNA cells/g. It was confirmed that drinking water supply systems (watershed-reservoir-drinking water treatment plant-household) might be a potential transmission route for mycobacteria.

1521 Bannantine, J.P., Bermudez, L.E.

No Holes Barred: Invasion of the Intestinal Mucosa by Mycobacterium avium subsp paratuberculosis
Infection and Immunity, (2013) 81, 3960-3965
The infection biology of Mycobacterium avium subsp. paratuberculosis has recently crystallized, with added details surrounding intestinal invasion. The involvement of pathogen-derived effector proteins such as the major membrane protein, oxidoreductase, and fibronectin attachment proteins have been uncovered. Mutations constructed in this pathogen have also shed light on genes needed for invasion. The host cell types that are susceptible to invasion have been defined, along with their transcriptional response. Recent details have given a new appreciation for the dynamic interplay between the host and bacterium that occurs at the outset of infection. An initial look at the global expression pathways of the host has shown a circumvention of the cell communication pathway by M. avium subsp. paratuberculosis, which loosens the integrity of the tight junctions. We now know that M. avium subsp. paratuberculosis activates the epithelial layer and also actively recruits macrophages to the site of infection. These notable findings are summarized along with added mechanistic details of the early infection model. We conclude by proposing critical next steps to further elucidate the process of M. avium subsp. paratuberculosis invasion .

1522 Lefrancois, L.H., Bodier, C.C., Cochard, T., Canepa, S., Raze, D., Lanotte, P., Sevilla, I.A., Stevenson, K., Behr, M.A., Locht, C., Biet, F.

Novel Feature of Mycobacterium avium subsp paratuberculosis, Highlighted by Characterization of the Heparin-Binding Hemagglutinin Adhesin
Journal of Bacteriology, (2013) 195, 4844-4853
Mycobacterium avium subsp. paratuberculosis comprises two genotypically defined groups, known as the cattle (C) and sheep (S) groups. Recent studies have reported phenotypic differences between M. avium subsp. paratuberculosis groups C and S, including growth rates, infectivity for macrophages, and iron metabolism. In this study, we investigated the genotypes and biological properties of the virulence factor heparin-binding hemagglutinin adhesin (HBHA) for both groups. In Mycobacterium tuberculosis, HBHA is a major adhesin involved in mycobacterium-host interactions and extrapulmonary dissemination of infection. To investigate HBHA in M. avium subsp. paratuberculosis, we studied hbhA polymorphisms by fragment analysis using the GeneMapper technology across a large collection of isolates genotyped by mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) and IS900 restriction fragment length polymorphism (RFLP-IS900) analyses. Furthermore, we analyzed the structure-function relationships of recombinant HBHA proteins of types C and S by heparin-Sepharose chromatography and surface plasmon resonance (SPR) analyses. In silico analysis revealed two forms of HBHA, corresponding to the prototype genomes for the C and S types of M. avium subsp. paratuberculosis. This observation was confirmed using GeneMapper on 85 M. avium subsp. paratuberculosis strains, including 67 strains of type C and 18 strains of type S. We found that HBHAs from all type C strains contain a short C-terminal domain, while those of type S present a long C-terminal domain, similar to that produced by Mycobacterium avium subsp. avium. The purification of recombinant HBHA from M. avium subsp. paratuberculosis of both types by heparin-Sepharose chromatography highlighted a correlation between their affinities for heparin and the lengths of their C-terminal domains, which was confirmed by SPR analysis. Thus, types C and S of M. avium subsp. paratuberculosis may be distinguished by the types of HBHA they produce, which differ in size and adherence properties, thereby providing new evidence that strengthens the genotypic differences between the C and S types of M. avium subsp. paratuberculosis.

1523 Trefz, P., Koehler, H., Klepik, K., Moebius, P., Reinhold, P., Schubert, J.K., Miekisch, W.

Volatile Emissions from Mycobacterium avium subsp paratuberculosis Mirror Bacterial Growth and Enable Distinction of Different Strains
Plos One, (2013) 8, Article Number: e76868 DOI: 10.1371/journal.pone.0076868
Published: OCT 8 2013-Control of paratuberculosis in livestock is hampered by the low sensitivity of established direct and indirect diagnostic methods. Like other bacteria, Mycobacterium avium subsp. paratuberculosis (MAP) emits volatile organic compounds (VOCs). Differences of VOC patterns in breath and feces of infected and not infected animals were described in first pilot experiments but detailed information on potential marker substances is missing. This study was intended to look for characteristic volatile substances in the headspace of cultures of different MAP strains and to find out how the emission of VOCs was affected by density of bacterial growth. One laboratory adapted and four field strains, three of MAP C-type and one MAP S-type were cultivated on Herrold's egg yolk medium in dilutions of 10(-0), 10(-2), 10(-4) and 10(-6). Volatile substances were pre-concentrated from the headspace over the MAP cultures by means of Solid Phase Micro Extraction (SPME), thermally desorbed from the SPME fibers and separated and identified by means of GC-MS. Out of the large number of compounds found in the headspace over MAP cultures, 34 volatile marker substances could be identified as potential biomarkers for growth and metabolic activity. All five MAP strains could clearly be distinguished from blank culture media by means of emission patterns based on these 34 substances. In addition, patterns of volatiles emitted by the reference strain were significantly different from the field strains. Headspace concentrations of 2-ethylfuran, 2-methylfuran, 3-methylfuran, 2-pentylfuran, ethyl acetate, 1-

methyl-1-H-pyrrole and dimethyldisulfide varied with density of bacterial growth. Analysis of VOCs emitted from mycobacterial cultures can be used to identify bacterial growth and, in addition, to differentiate between different bacterial strains. VOC emission patterns may be used to approximate bacterial growth density. In a perspective volatile marker substances could be used to diagnose MAP infections in animals and to identify different bacterial strains and origins.

1524 Mensikova, M., Stepanova, H., Faldyna, M.

Interleukin-17 in veterinary animal species and its role in various diseases: A review
Cytokine, (2013) 64, 11-17
Interleukin 17 (IL-17) as one of the pro-inflammatory cytokines is a very important player in the immune response to many pathogens and seems to play a role also in certain chronic and autoimmune diseases. Many studies showing the importance of this cytokine were conducted on murine models and human patients. In recent years, some experiments with other animals in which interleukin-17 was measured were carried out. This review is focused on the findings that have been observed and described in important veterinary species of animals.

1525 Smith, E.J. , Thompson, A.P., O'Driscoll, A., Clarke, D.J.

Pathogenesis of adherent-invasive Escherichia coli
Future Microbiology, (2013) 8, 1289-1300
The etiology of Crohns disease (CD) is complex and involves both host susceptibility factors (i.e., the presence of particular genetic alleles) and environmental factors, including bacteria. In this regard, adherent-invasive Escherichia coli (AIEC), have recently emerged as an exciting potential etiological agent of CD. AIEC are distinguished from commensal strains of E. coli through their ability to adhere to and invade epithelial cells and replicate in macrophages. Recent molecular analyses have identified genes required for both invasion of epithelial cells and replication in the macrophage. However, these genetic studies, in combination with recent genome sequencing projects, have revealed that the pathogenesis of this group of bacteria cannot be explained by the presence of AIEC-specific genes. In this article, we review the role of AIEC as a pathobiont in the pathology of CD. We also describe the emerging link between AIEC and autophagy, and we propose a model for AIEC pathogenesis.

1526 Basler, T., Brumshagen, C., Beineke, A., Goethe, R., Baumer, W.

Mycobacterium avium subspecies impair dendritic cell maturation
Innate Immunity, (2013) 19, 451-461
Mycobacterium avium ssp. paratuberculosis (MAP) causes Johne's disease, a chronic, granulomatous enteritis of ruminants. Dendritic cells (DC) of the gut are ideally placed to combat invading mycobacteria; however, little is known about their interaction with MAP. Here, we investigated the interaction of MAP and the closely related M. avium ssp. avium (MAA) with murine DC and the effect of infected macrophages on DC maturation. The infection of DC with MAP or MAA induced DC maturation, which differed to that of LPS as maturation was accompanied by higher production of IL-10 and lower production of IL-12. Treatment of maturing DC with supernatants from mycobacteria-infected macrophages resulted in impaired DC maturation, leading to a semi-mature, tolerogenic DC phenotype expressing low levels of MHCII, CD86 and TNF- after LPS stimulation. Though the cells were not completely differentiated they responded with an increased IL-10 and a decreased IL-12 production. Using recombinant cytokines we provide evidence that the semi-mature DC phenotype results

from a combination of secreted cytokines and released antigenic mycobacterial components of the infected macrophage. Our results indicate that MAP and MAA are able to subvert DC function directly by infecting and indirectly via the milieu created by infected macrophages.

1527 Nagata, R., Kawaji, S., Mori, Y.

Use of enoyl coenzyme A hydratase of Mycobacterium avium subsp paratuberculosis for the serological diagnosis of Johne's disease
Veterinary Immunology and Immunopathology, (2013) 155, 253-258
Johne's disease (JD), caused by Mycobacterium avium subspecies paratuberculosis (MAP), remains difficult to control because of the lack of specific and sensitive diagnostic tests. In order to improve the specificity of sero-diagnosis for JD, the phage display library derived from genomic DNA of MAP was immunoscreened to identify novel antigenic targets. We selected a clone using antibodies from MAP experimentally infected cattle, and annotated its coding sequence as MAP1197 in the MAP genome, which encoded "echA12_2" in the MAP protein (Map-echA) belonging to Enoyl-CoA hydratase, known as a crotonase enzyme. The Map-echA was expressed in Esherichia coil and purified as a histidine-tag recombinant protein (rMap-echA), and the diagnostic potential of the protein was further evaluated by enzyme-linked immunosorbent assays (ELISA). Antibody responses to rMap-echA were higher in MAP-infected cattle than in uninfected cattle. The specificity of the Map-echA ELISA was also confirmed by evaluation with hyper-immune sera against various kinds of Mycobacterium species. Furthermore, in all experimentally infected cattle the antibody against rMap-echA was detected 2-7 months earlier than by a commercially available ELISA kit. These results suggested that Map-echA can be used as a specific and sensitive serological diagnostic antigen for the detection of MAP infection. (C) 2013 Elsevier B.V. All rights reserved.

1528 Thakur, A., Riber, U., Davis, W.C., Jungersen, G.

Increasing the ex vivo antigen-specific IFN-gamma production in subpopulations of T cells and NKp46+ cells by anti-CD28, anti-CD49d and recombinant IL-12 costimulation in cattle vaccinated with recombinant proteins from Mycobacterium avium subspecies paratuberculosis
Veterinary Immunology and Immunopathology, (2013) 155, 276-283
T cells, which encounter specific antigen (Ag), require additional signals to mount a functional immune response. Here, we demonstrate activation of signal 2, by anti-CD28 mAb (aCD28) and other costimulatory molecules (aCD49d, aCD5), and signal 3, by recombinant IL-12, enhance Ag-specific IFN-gamma secretion by CD4, CD8, gamma delta T cells and NK cells. Age matched male jersey calves, experimentally infected with Mycobacterium avium subsp. paratuberculosis (MAP), were vaccinated with a cocktail of recombinant MAP proteins or left unvaccinated. Vaccine induced ex vivo recall responses were measured through Ag-specific IFN-gamma production by ELISA and flow cytometry. There was a significant increase in production of IFN-gamma by T cell subsets or NKp46+ cells cultured in the presence of Ag and aCD28/aCD49d. The increase was accompanied by an increase in the integrated median fluorescence intensity (iMFI) of activated T cells. Addition of rIL-12 induced a significant additive effect leading to a maximum increase in responder frequency of Ag-specific T cell subsets or NKp46+ cells with a heavy bias toward IFN-gamma production by CD4 T cells. We provide the first description of using aCD28/aCD49d costimulation to potentiate an Agspecific increase in the production of IFN-gamma in bovine immunology. The study also shows the degree of signaling in T cells is regulated by the costimulatory environment. (C) 2013 Elsevier B.V. All rights reserved.

1529 Souza, C., Davis, W.C., Eckstein, T.M., Sreevatsan, S., Weiss, D.J.

Mannosylated Lipoarabinomannans from Mycobacterium Avium Subsp Paratuberculosis Alters the Inflammatory Response by Bovine Macrophages and Suppresses Killing of Mycobacterium Avium Subsp Avium Organisms
Plos One, (2013) 8, Article Number: e75924 DOI: 10.1371/journal.pone.0075924
Published: SEP 30 2013-Analysis of the mechanisms through which pathogenic mycobacteria interfere with macrophage activation and phagosome maturation have shown that engagement of specific membrane receptors with bacterial ligands is the initiating event. Mannosylated lipoarabinomannan (Man-LAM) has been identified as one of the ligands that modulates macrophage function. We evaluated the effects of Man-LAM derived from Mycobacterium avium subsp. paratuberculosis (MAP) on bovine macrophages. Man-LAM induced a rapid and prolonged expression of IL-10 message as well as transient expression of TNF-alpha. Preincubation with Man-LAM for up to 16 h did not suppress expression of IL-12 in response to interferon-gamma. Evaluation of the effect of Man-LAM on phagosome acidification, phagosome maturation, and killing of Mycobacterium avium subsp. avium (MAA) showed that preincubation of macrophages with Man-LAM before addition of MAA inhibited phagosome acidification, phagolysosome fusion, and reduced killing. Analysis of signaling pathways provided indirect evidence that inhibition of killing was associated with activation of the MAPK-p38 signaling pathway but not the pathway involved in regulation of expression of IL-10. These results support the hypothesis that MAP Man-LAM is one of the virulence factors facilitating survival of MAP in macrophages.

1530 Jolly, A., Morsella, C., Bass, L., Fiorentino, M.A., Paolicchi, F.A., Mundo, S.L.

Bovine response to lipoarabinomannan vaccination and challenge with Mycobacterium paratuberculosis
Brazilian Journal of Microbiology, (2013) 44, 511-514
This study aimed to evaluate the immune response in bovines following immunization with a mycobaterial Lipoarabinomannan extract (LAMe) and the effect of Map challenge. LAMe vaccine induced specific antibody levels that diminished after the challenge and affected Map excretion at least for 100 days thereafter.

1531 Rani, P.S., Babajan, B., Tulsian, N.K., Begum, M., Kumar, A., Ahmed, N.

Mycobacterial Hsp65 potentially cross-reacts with autoantibodies of diabetes sera and also induces (in vitro) cytokine responses relevant to diabetes mellitus
Molecular Biosystems, (2013) 9, 2932-2941
Diabetes mellitus is a multifactorial disease and its incidence is increasing worldwide. Among the two types of diabetes, type-2 accounts for about 90% of all diabetic cases, whereas type-1 or juvenile diabetes is less prevalent and presents with humoral immune responses against some of the autoantigens. We attempted to test whether the sera of type-1 diabetes patients cross-react with mycobacterial heat shock protein 65 (Hsp65) due to postulated epitope homologies between mycobacterial Hsp65 and an important autoantigen of type-1 diabetes, glutamic acid decarboxylase-65 (GAD65). In our study, we used either recombinant mycobacterial Hsp65 protein or synthetic peptides corresponding to some of the potential epitopes of mycobacterial Hsp65 that are shared with GAD65 or human Hsp60, and a control peptide sourced from mycobacterial Hsp65 which is not shared with GAD65, Hsp60 and other autoantigens of type-1 diabetes. The indirect ELISA results indicated that both type-1 diabetes and type-2 diabetes sera cross-react with conserved mycobacterial Hsp65 peptides and recombinant mycobacterial Hsp65 protein but do not do so with the control peptide. Our results suggest that cross-reactivity of mycobacterial Hsp65 with autoantibodies of diabetes sera could be due to the presence of significantly conserved peptides between mycobacterial Hsp65 and human Hsp60 rather than between mycobacterial Hsp65 and GAD65. The

treatment of human peripheral blood mononuclear cells (PBMCs) with recombinant mycobacterial Hsp65 protein or the synthetic peptides resulted in a significant increase in the secretion of cytokines such as IL-1 beta, IL-8, IL-6, TNF-alpha and IL-10. Taken together, these findings point towards a dual role for mycobacterial Hsp65: in inducing autoimmunity and in inflammation, the two cardinal features of diabetes mellitus.

1532 Frau, J., Cossu, D., Coghe, G., Lorefice, L., Fenu, G., Melis, M., Paccagnini, D., Sardu, C., Murru, M.R., Tranquilli, S. , Marrosu, M.G., Sechi, L.A., Cocco, E.

Mycobacterium avium subsp paratuberculosis and multiple sclerosis in Sardinian patients: epidemiology and clinical features
Multiple Sclerosis Journal, (2013) 19, 1437-1442
Background:Mycobacterium avium subspecies paratuberculosis (MAP) is an infectious factor recently found in association with multiple sclerosis (MS) in Sardinia. Objectives: The objectives of this study were to confirm this association and evaluate its role in clinical features. Methods: A total of 436 patients and 264 healthy controls (HCs) were included. We examined the blood of each individual for MAPDNA and MAP2694 antibodies using IS900-specific PCR and ELISA, respectively. Differences in MAP presence between the MS group and HCs were evaluated. In MS patients, we considered: gender, age, age at onset, duration of disease, course, EDSS, therapy, relapse/steroids at study time, and oligoclonal bands (OBs). Results: MAPDNA and MAP2694 antibodies were detected in 68 MS and six HCs (p = 1.14 x 10(-11)), and 123 MS and 10 HCs (p = 2.59 x 10(-23)), respectively. OBs were found with reduced frequency in MAP-positive patients (OR = 0.52; p = 0.02). MAP2694 antibodies were detected more in patients receiving MS treatments (OR = 2.26; p = 0.01), and MAPDNA in subjects on steroids (OR = 2.65; p = 0.02). Conclusion: Our study confirmed the association of MAP and MS in Sardinia. The low OB frequency in MAP patients suggests a peripheral role as a trigger in autoimmunity. MAP positivity might be influenced by steroids and MS therapy. Studies in other populations are needed to confirm the role of MAP in MS.

1533 Teixeira, A.G.V., Bicalho, M.L.S., Machado, V.S., Oikonomou, G., Kacar, C., Foditsch, C., Young, R., Knauer, W.A., Nydam, D.V., Bicalho, R.C.

Heat and ultraviolet light treatment of colostrum and hospital milk: Effects on colostrum and hospital milk characteristics and calf health and growth parameters
Veterinary Journal, (2013) 197, 175-181
The aim of this study was to evaluate the effects of different physical treatments of bovine colostrum and hospital milk on milk bacteriology, immunoglobulin G (IgG) and lactoferrin concentrations, calf serum IgG concentrations and calf health, growth and survivability. Pooled colostrum samples (n = 297) were heat treated (HTC; 63 degrees C for 60 min), exposed to ultraviolet light (UVC; 45 J/cm(2)) or untreated ('raw', RC). Hospital milk (n = 712) was subjected to high temperature short time pasteurization (HTST; 72 degrees C for 15 s), ultraviolet light irradiation (UVH; 45 J/cm(2)) or was untreated. Neonatal Holstein heifer calves (n = 875) were randomly enrolled (309 HTC, 285 UVC, 281 RC) and block randomized (by colostrum treatment) into hospital milk treatments HTST (n = 449) or UVH (n = 426). HTC was more effective than UVC and HTST was more effective than UVH in reducing bacterial counts. IgG and lactoferrin concentrations were significantly lower in HTC and UVC than in RC. Lactoferrin concentrations were significantly lower in HTST than in UVH or untreated hospital milk. There were no significant differences in serum IgG concentrations among calves fed HTC, UVC or RC. Colostrum and hospital milk treatments did not have any significant effect on calf body weight gain, survivability, or frequency of diarrhea or pneumonia. (C) 2013 Elsevier Ltd. All rights reserved.

New publications in the CROHN'S DISEASE AND PARATUBERCULOSIS database (846-854)

846 Corridoni, D., Kodani, T., Rodriguez-Palacios, A., Pizarro, T.T., Xin, W., Nickerson, K.P., McDonald, C., Ley, K.F., Abbott, D.W., Cominelli, F.

Dysregulated NOD2 predisposes SAMP1/YitFc mice to chronic intestinal inflammation
Proceedings of the National Academy of Sciences of the United States of America, (2013) 110, 16999-17004
Nucleotide-binding oligomerization domain-containing 2 (NOD2) is an intracellular receptor that plays an essential role in innate immunity as a sensor of a component of the bacterial cell wall, muramyl dipeptide (MDP). Crohn's disease (CD)-associated NOD2 variants lead to defective innate immune responses, including decreased NF-kappa B activation and cytokine production. We report herein that SAMP1/YitFc (SAMP) mice, which develop spontaneous CD-like ileitis in the absence of NOD2 genetic mutations, fail to respond to MDP administration by displaying decreased innate cytokine production and dysregulated NOD2 signaling compared with parental AKR control mice. We show that, unlike in other mouse strains, in vivo administration of MDP does not prevent dextran sodium sulfate-induced colitis in SAMP mice and that the abnormal NOD2 response is specific to the hematopoietic cellular component. Moreover, we demonstrate that MDP fails to enhance intracellular bacterial killing in SAMP mice. These findings shed important light on the initiating molecular events underlying CD-like ileitis.

847 Rubino, S.J. , Magalhaes, J.G., Philpott, D., Bahr, G.M., Blanot, D., Girardin, S.E.

Identification of a synthetic muramyl peptide derivative with enhanced Nod2 stimulatory capacity
Innate Immunity, (2013) 19, 493-503
Muramyl peptides (MPs) represent the building blocks of bacterial peptidoglycan, a critical component of bacterial cell walls. MPs are well characterized for their immunomodulatory properties, and numerous studies have delineated the role of MPs or synthetic MP analogs in host defense, adjuvanticity and inflammation. More recently, Nod1 and Nod2 have been identified as the host sensors for specific MPs, and, in particular, Nod2 was shown to detect muramyl dipeptide (MDP), a MP found in both Gram-positive and Gram-negative bacterial cell walls. Because mutations in Nod2 are associated with the etiology of Crohn's disease, there is a need to identify synthetic MP analogs that could potentiate Nod2-dependent immunity. Here, we analyzed the Nod2-activating property of 36 MP analogs that had been tested previously for their adjuvanticity and anti-infectious activity. Using a luciferase-based screen, we demonstrate that addition of a methyl group to the second amino acid of MDP generates a MDP derivative with enhanced Nod2-activating capacity. We further validated these results in murine macrophages, human dendritic cells and invivo. These results offer a basis for the rational development of synthetic MPs that could be used in the treatment of inflammatory disorders that have been associated with Nod2 dysfunction, such as Crohn's disease.

848 Lin, Z.W., Hegarty, J.P., John, G., Berg, A., Wang, Z., Sehgal, R., Pastor, D.M., Wang, Y.H., Harris, L.R., Poritz, L.S., Schreiber, S., Koltun, W.A.

NOD2 Mutations Affect Muramyl Dipeptide Stimulation of Human B Lymphocytes and Interact with Other IBD-Associated Genes
Digestive Diseases and Sciences, (2013) 58, 2599-2607
Background Genetic and functional studies have associated variants in the NOD2/CARD15 gene with Crohn's disease. Aims This study aims to replicate the association of three common NOD2 mutations with Crohn's disease, study its effect on NOD2 expression in B cells and its

interaction with other IBD-associated genes. Methods A total of 294 IBD patients (179 familial IBD, 115 sporadic IBD) and 298 unrelated healthy controls were from central Pennsylvania. NOD2 mutations were analyzed by primer-specific amplification, PCR based-RFLP, and validated with the ABI SNPlex(M) genotyping system. Gene-gene interaction was studied using a statistical model for epistasis analysis. Results Three common NOD2 mutations are associated with Crohn's disease (p = 5.08 x 10(-7), 1.67 x 10(-6), and 1.87 x 10(-2) for 1007fs, R720W, and G908R, respectively), but not with ulcerative colitis (p = 0.1046, 0.1269, and 0.8929, respectively). For IBD overall, 1007finsC (p = 4.4 x 10(-5)) and R720W (p = 9.24 x 10(-5)) were associated with IBD, but not G908R (p = 0.1198). We revealed significant interactions of NOD2 with other IBD susceptibility genes IL23R, DLG5, and OCTN1. We discovered that NOD2 was expressed in both normal human peripheral blood B cells and in EBV-transformed B cell lines. Moreover, we further demonstrated that muramyl dipeptide (MDP) stimulation of B lymphocytes up-regulated expression of NF-kappa B-p50 mRNA. Conclusion NOD2 is expressed in peripheral B cells, and the up-regulation of NOD2 expression by MDP was significantly impaired by NOD2 mutations. The finding suggests a possible role of NOD2 in the immunological response in IBD pathogenesis.

849 Rickard, D.J., Sehon, C.A., Kasparcova, V. , Kallal, L.A., Zeng, X., Montoute, M.N., Chordia, T., Poore, D.D., Li, H., Wu, Z.N., Eidam, P.M., Haile, P.A., Yu, J., Emery, J.G., Marquis, R.W., Gough, P.J., Bertin, J.

Identification of Benzimidazole Diamides as Selective Inhibitors of the Nucleotide-Binding Oligomerization Domain 2 (NOD2) Signaling Pathway
Plos One, (2013) 8, Article Number: e69619 DOI: 10.1371/journal.pone.0069619
Published: AUG 1 2013-NOD2 is an intracellular pattern recognition receptor that assembles with receptor-interacting protein (RIP)-2 kinase in response to the presence of bacterial muramyl dipeptide (MDP) in the host cell cytoplasm, thereby inducing signals leading to the production of pro-inflammatory cytokines. The dysregulation of NOD2 signaling has been associated with various inflammatory disorders suggesting that small-molecule inhibitors of this signaling complex may have therapeutic utility. To identify inhibitors of the NOD2 signaling pathway, we utilized a cell-based screening approach and identified a benzimidazole diamide compound designated GSK669 that selectively inhibited an MDP-stimulated, NOD2-mediated IL-8 response without directly inhibiting RIP2 kinase activity. Moreover, GSK669 failed to inhibit cytokine production in response to the activation of Toll-like receptor (TLR)-2, tumor necrosis factor receptor (TNFR)-1 and closely related NOD1, all of which share common downstream components with the NOD2 signaling pathway. While the inhibitors blocked MDP-induced NOD2 responses, they failed to block signaling induced by NOD2 over-expression or single stranded RNA, suggesting specificity for the MDP-induced signaling complex and activator-dependent differences in NOD2 signaling. Investigation of structure-activity relationship allowed the identification of more potent analogs that maintained NOD2 selectivity. The largest boost in activity was achieved by N-methylation of the C2-ethyl amide group. These findings demonstrate that the NOD2 signaling pathway is amenable to modulation by small molecules that do not target RIP2 kinase activity. The compounds we identified should prove useful tools to investigate the importance of NOD2 in various inflammatory processes and may have potential clinical utility.

850 Antosz, H., Osiak, M.

NOD1 and NOD2 receptors: integral members of the innate and adaptive immunity system
Acta Biochimica Polonica, (2013) 60, 351-360
NOD-like proteins (NLR) are a specialized group of intracellular receptors, which constitute an essential component of the host innate immune system. They were discovered more than a decade ago, but research on this particular class of microbial detectors is still ongoing to allow

for a better understanding of the mechanisms, recognition of microorganisms, transmission of signals, and carrying out the activation of inflammatory signaling pathways. In this review, we discuss the construction of NOD1 and NOD2 receptors, their functions, and significance in the pathogenesis of inflammatory diseases in humans.

851 Willison, H.J., Goodyear, C.S.

Glycolipid antigens and autoantibodies in autoimmune neuropathies
Trends in Immunology, (2013) 34, 453-459
Autoantibodies to glycans present on glycolipids mediate the postinfectious paralytic disease, Guillain-Barre syndrome (GBS). These glycans are also found on lipooligosaccharides (LOSs) of GBS-inducing microbes, suggesting molecular mimicry as a mechanism for disease induction. How B lymphocyte tolerance to self-glycans is regulated during the initiation phase of the disease is currently under investigation. The discovery of antigly-colipid antibodies that bind to heteromeric glycolipid complexes has generated new insights in this field. Heteromeric complexes are structurally distinct glycolipids that interact to form new molecular shapes capable of either enhancing or attenuating recognition by auto-antibodies. Although the principles emerging from this phenomenon have a substantial impact on diagnostics methods, they also raise intriguing questions about the diversity of innate antibody repertoires, mechanisms of tolerance, and autoantibody targeting of neural membranes.

852 Kuuliala, K. , Lappalainen, M., Turunen, U., Puolakkainen, P., Kemppainen, E., Siitonen, S., Repo, H., Mustonen, H.

Detection of muramyl dipeptide-sensing pathway defects in monocytes of patients with Crohn's disease using phospho-specific whole blood flow cytometry
Scandinavian Journal of Clinical & Laboratory Investigation, (2013) 73, 494-502
Peripheral blood mononuclear cells of Crohn's disease (CD) patients with the common 1007fs mutation of the caspase recruitment domain-containing 15/nucleotide-binding oligomerization domain-containing 2 (CARD15/NOD2) gene show impaired nuclear factor kappa B (NF-kappa B) activation in response to muramyl dipeptide (MDP), as determined by Western blotting. We applied phospho-specific flow cytometry to examine NF-kappa B and p38 activation in whole blood monocytes of 16 CD patients with or without the 1007fs and previously described rare mutations of the CARD15 gene, and healthy reference subjects. Aliquots of whole blood were supplemented with MDP (0-1000 ng/mL), incubated for 10-40 min and processed for flow cytometry. Bacterial lipopolysaccharide (LPS) was used as a positive control agonist. We found that NF-kappa B and p38 phosphorylation induced by MDP was not detectable in monocytes of patients homozygous for the CARD15 1007fs mutation, while those induced by LPS were normal. We also determined MDP-induced NF-kappa B phosphorylation levels in nuclear extracts of mononuclear cells separated from blood using enzyme-linked immunosorbent assay (ELISA), and observed that the levels decreased in a 1007fs mutation-dose dependent manner. We conclude that phospho-specific whole blood flow cytometry provides a means to study phosphorylation of NF-kappa B and p38 in clinical samples and can be applied to screening of CD patients homozygous for the CARD15 1007fs mutation.

853 Spalinger, M.R., Lang, S., Vavricka, S.R., Fried, M., Rogler, G., Scharl, M.

Protein Tyrosine Phosphatase Non-Receptor Type 22 Modulates NOD2-Induced Cytokine Release and Autophagy
Plos One, (2013) 8, Article Number: e72384 DOI: 10.1371/journal.pone.0072384
Published: AUG 26 2013-Background: Variations within the gene locus encoding protein

tyrosine phosphatase non-receptor type 22 (PTPN22) are associated with the risk to develop inflammatory bowel disease (IBD). PTPN22 is involved in the regulation of T- and B-cell receptor signaling, but although it is highly expressed in innate immune cells, its function in other signaling pathways is less clear. Here, we study whether loss of PTPN22 controls muramyl-dipeptide (MDP)-induced signaling and effects in immune cells. Material & Methods: Stable knockdown of PTPN22 was induced in THP-1 cells by shRNA transduction prior to stimulation with the NOD2 ligand MDP. Cells were analyzed for signaling protein activation and mRNA expression by Western blot and quantitative PCR; cytokine secretion was assessed by ELISA, autophagosome induction by Western blot and immunofluorescence staining. Bone marrow derived dendritic cells (BMDC) were obtained from PTPN22 knockout mice or wild-type animals. Results: MDP-treatment induced PTPN22 expression and activity in human and mouse cells. Knockdown of PTPN22 enhanced MDP-induced activation of mitogen-activated protein kinase (MAPK)-isoforms p38 and c-Jun N-terminal kinase as well as canonical NF-kappa B signaling molecules in THP-1 cells and BMDC derived from PTPN22 knockout mice. Loss of PTPN22 enhanced mRNA levels and secretion of interleukin (IL)-6, IL-8 and TNF in THP-1 cells and PTPN22 knockout BMDC. Additionally, loss of PTPN22 resulted in increased, MDP-mediated autophagy in human and mouse cells. Conclusions: Our data demonstrate that PTPN22 controls NOD2 signaling, and loss of PTPN22 renders monocytes more reactive towards bacterial products, what might explain the association of PTPN22 variants with IBD pathogenesis.

854 Lorton, D., Bellinger, D.L., Schaller, J.A., Shewmaker, E., Osredkar, T., Lubahn, C.

Altered Sympathetic-to-Immune Cell Signaling via beta(2)-Adrenergic Receptors in Adjuvant Arthritis
Clinical & Developmental Immunology, (2013) none, Article Number: 764395 DOI: 10.1155/2013/764395 Published: 2013-Adjuvant-induced arthritic (AA) differentially affects norepinephrine concentrations in immune organs, and in vivo beta-adrenergic receptor (beta-AR) agonist treatment distinctly regulates ex vivo cytokine profiles in different immune organs. We examined the contribution of altered beta-AR functioning in AA to understand these disparate findings. Twenty-one or 28 days after disease induction, we examined beta(2)-AR expression in spleen and draining lymph nodes (DLNs) for the arthritic limbs using radioligand binding and western blots and splenocyte beta-AR-stimulated cAMP production using enzyme-linked immunoassay (EIA). During severe disease, beta-AR agonists failed to induce splenocyte cAMP production, and beta-AR affinity and density declined, indicating receptor desensitization and downregulation. Splenocyte beta(2)-AR phosphorylation (p beta(2)-AR) by protein kinase A (p beta(2)-AR(PKA)) decreased in severe disease, and p beta(2)-AR by G protein-coupled receptor kinases (p beta(2)-AR(GRK)) increased in chronic disease. Conversely, in DLN cells, p beta(2)-AR(PKA) rose during severe disease, but fell during chronic disease, and p beta(2)-AR(GRK) increased during both disease stages. A similar p beta(2)-AR pattern in DLN cells with the mycobacterial cell wall component of complete Freund's adjuvant suggests that pattern recognition receptors (i.e., toll-like receptors) are important for DLN p beta(2)-AR patterns. Collectively, our findings indicate lymphoid organ- and disease stage-specific sympathetic dysregulation, possibly explaining immune compartment-specific differences in beta(2)-AR-mediated regulation of cytokine production in AA and rheumatoid arthritis.

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